

Amendments to the Specification

On page 29, please replace the paragraph under “E. Analysis of Protein from Transformed Tobacco Plants” with the following:

Protein was extracted from transformed tobacco leaf tissues by homogenization with a Ten-Broek ground glass homogenizer (clearance 0.15 mm) in five volumes of buffer containing twenty millimolar (20mM) sodium phosphate, pH 7.0, one hundred fifty millimolar (150mM) sodium chloride, twenty millimolar (20mM) sodium ascorbate, one-tenth percent (0.1%) Triton X-100, and five tenths millimolar (0.5mM) PMSF, at four degrees Celsius (4°C). The homogenate was centrifuged at one thousand times gravity (1000XG) for five minutes and the supernatant centrifuged at twenty-seven thousand times gravity (27,000XG) for fifteen minutes. The 27,000XG supernatant was then centrifuged at one hundred thousand times gravity (100,000XG) for one hour and the pellet resuspended in extraction buffer. The protein in the different fractions was measured by the Coomassie dye-binding assay (Bio-Rad). HBsAg protein was assayed by the AUSZYME Monoclonal kit (Abbott Laboratories, Abbott Park, IL) using the positive control, HBsAg derived from human serum, as the standard. The positive control was diluted to give HBsAg protein levels of nine hundredths to one and eight tenths nanograms (.09-1.8 ng) per assay. After color development, the absorbance at four hundred ninety-two nanometers (492 nm) was read and a linear relationship was found. As seen in Figure 6B, the ~~wild-type~~ wild-type control plant contained no detectable HBsAg protein (Column 1); fairly low levels of HBsAg protein were observed, ranging from three to ten nanograms per milligram (3-10ng/mg) soluble protein for the pHB101 construct (Columns 2 through 6); and from twenty-five to sixty-five nanograms per milligram (25-65 ng/mg) for the pHB102 construct (Columns 7 through 9). The reaction was specific because the wild-type tobacco showed no detectable HBsAg protein. HBsAg from human serum and recombinant HBsAg (rHBsAg) from plasmid-transformed yeast occur as approximately twenty nanometer (20nm) spherical particles consisting of protein embedded in a phospholipid bilayer. Ninety-five percent of the rHBsAg in the 27,000XG supernatants of transgenic tobacco

leaf extracts pelleted at 2000,000XG for thirty minutes. This suggested a particle form. Thus, evidence was sought to ascertain if rHBsAg in tobacco existed as particles.